Study on biodegradated ability of thirteen edible fungi to straw

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Abstract: The biodegradated abilities of 13 edible fungi to straw were studied. The results showed that all the experimental fungi except *Tricholama mongolicum* had definite biodegradated abilities to the lignin and cellulose of straw. The Ideal fungus for straw degradation was screened out as *Pleurotus ostreatus*, which showed a higher degradation ability for lignin (17.86%) and lower degradation rate for cellulose (2.24%), with a Selection Factor (SF) of 7.97. The degradation rates of lignin and cellulose for other fungi ranged from 2.30% to 16.54% and 5.60% to 17.32%, respectively, and the SF was very low in range of 0.14 to 2.24. The ratio of colony's diameters to the color-zone (d_1/d_2) and SF are negative correlation, with a correlation coefficient of -0.1476.
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Introduction

With the continuous exploitation of industrial resources, straws haven't already been a main energy source. The content of cell wall in the dry matter of straws is the highest and this matter cannot be degraded easily, which mainly consists of cellulose, hemicellulose, and lignin, and then its surface attaches to mineral, cutin and suberin etc.. Moreover lignin, not a carbohydrate, is a phene-aldehyde and propane polymer that is composed of cutin, monosaccharide, protein, and silicate. Lignin restrains the assimilation and absorption of cellulose, thereby leads to a lower utilization rate as feed. Only to degrade or partly degrade the lignin of straws could increase the utilization rate of straw (Ding *et al.* 1997).

In nature, the degradation of lignin is mainly conducted by microorganisms. The biodegradation of lignin mainly utilized exocellular enzyme coming from microorganism (mainly including the lignin oxidase family, which have stronger catalyze oxidative action on lignin), making lignin decompose and transform gradually. Different microorganism has different degradation ability to lignin. At present, the research mainly concentrated on the possibility of white rot fungi applying to industry. Edible fungi as a mainly species of fungi have a closely relation with mankind, also have definite degradation ability to lignin and get more and more concerns (Wang *et al.* 1998). The source of straws is very abundant in rural area, but its utilization rate is very low, most only as feed and fuel, moreover as feed it cannot be utilized effectively; and as fuel it easily causes environmental pollution. Planting edible fungi is an effective approach to solve this problem. The straw is a good material for planting edible fungi. The immanent fabric among cell of straw is destroyed easily by edible fungi, and cellulose and hemicellulose will expose directly to the enzyme that the edible fungi excreted. In addition, by the action of edible fungi, the straw can be transformed into more saccharide and protein, which is assimilated more easily by livestock. At present, there are a few of reports about the utilization of the scrap of edible fungi planting, which may as feed and fuel and can extract some useful substance (Xu 1997). Thus, through planting edible fungi, we can make use of the advantages of straw to get good economic, social and environmental benefit.

This paper mainly studied the biodegradation ability of some edible fungi to straw, and laid a base for further research.

Materials and methods

Materials

Strains

Tricholama mongolicum Imai are collected from Inner Mongolia of China.

Agaricus bisporus (Lange) sing, *Agarixus blazei* Murr, and *Coprinus comatus* (mull.: Fr.) Gray come from Heilongjiang Institute of Microorganism.

Pleurotus ostreatus (Jacq.: Fr.) Quel., *P. cetrinipileatus* Sing., *P. cornucopiae* (Pual.: Pers.) Roll., *P. eryngii* var. *nebrodensis* Inzenga., *Hericium erinaceus* (Bull.) Pers., *Pholiota nameko* (T. Ito.) S Ito et Imai., *Flammulina velutipes* (Curt.: Fr) Sing. (yellow), *F. velutipes* (Curt.: Fr) Sing. (white), and *Hohenbuehella serotina* (Schran.: Fr) Sing. come from Mudanjiang Edible Fungi Factory, Heilongjiang Province of China.

Straw powder

Straws are collected from ACheng farm, Heilongjiang

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Province. It is air-dry, crushed with pulverizer, and filtrated with 60 meshes, finally made the straw powder.

Culture media

Culture medium Ⅰ: 2.0% 60-mesh straw powder, 0.1% sucrose, 0.1% peptone, 0.02% guaiacol, 2.0% agar.

Culture medium Ⅱ: 2.0% 60-mesh straw powder, 0.1% sucrose, 0.02% guaiacol, 2.0% agar.

Culture medium of malt extract (L^{-1}): malt extract 10 g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4$ 0.01 g, K_2HPO_4 1.0 g, distilled water 1 000 mL.

Culture medium of inorganic salt (L^{-1}): glucose 20 g, NH_4NO_3 0.5 g, KH_2PO_4 1.0 g, $Na_2HPO_4 \cdot 12H_2O$ 0.4 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, VB_1 0.1 mg, $CaCl_2$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.1 mg, adjusting pH =5.0 with H_2SO_4.

PDA culture medium (L^{-1}): potato 200 g, glucose 20 g, agar 20 g (Zhang *et al.* 2000)

Methods

Screening strains

Nishida *et al.* considered that the microorganism could produce color-zone on the culture medium of guaiacol plate and had the ability of degradation lignin (E.de Jong *et al.* 1992). There are types of color-zone: one is on the outside of colony ($d_1/d_2<1$), the other is inside of colony ($d_1/d_2>1$). Ander and Eriksson's experiment indicated that the ratio of d_1/d_2 can be regarded as the warrant whether the fungus has the ability of degrading lignin, if $d_1/d_2<1$, this fungus can selectively degrade lignin, if $d_1/d_2>1$, this fungus can degrade cellulose firstly.

The strains of edible fungi were inoculated on the plate of culture medium Ⅰ, and then the plate was put in the constant temperature incubator of 25°C for 7 days. The strains that can produce color-zone were selected, and then the pure strain was inoculated on the PDA culture slop at the temperature of 25°C for 7 days.

Experiment of color-zone

The strains producing color-zone were inoculated on the plate of culture medium Ⅱ, at 25°C for 7 days. The diameters of colony (d_1) and color-zone (d_2) were measured, and then the ratio of colony and color-zone (d_1/d_2) was calculated.

Experiment of degrading lignin

Using guaiacol culture medium could only determine whether the strain might degrade lignin, as for the degradation ability must be confirmed through the experiment of lignin degradation. Through the experiment of lignin degradation, we could know the degradation abilities of different strain to lignin and cellulose, and the ratio of the lost weight of lignin and cellulose (Selection Factors) were different and they could be as the basis of screening out the highly effective degradation fungus to lignin.

(1) Liquid culture of strain: the strain was inoculated on the plate of PDA at 25°C for 7 days, and then taken equal mycelia in culture medium of melt extract, shaken (115 $r \cdot min^{-1}$) culture at 30°C for 7 days.

(2) Preparation of the straw culture medium: Weigh up 2 g 60-mesh straw powder. After extracted by the mixture of grain alcohol and benzene (GB2677.7-81), they were put into a 250-mL conical flask, then, 5-mL inorganic salt culture medium was put into the flask, making the extracted straw powder wet entirely, and sterilized at 121°C for 20 min.

(3) The sterilized glass injector (10 mL) was used to get 10 mL mycelia suspend liquid, and then the straw powder culture was added and cultured at 25°C for 15 days.

Determination of biodegradation ratio and calculation of the selection factor

The contents of lignin and total cellulose of straw powder inoculated before and after were analyzed firstly. The straw powder was put into drying-oven under 105°C, and then put into mortar to grind for content.

(1) Determination of Klason lignin: The sample was transferred carefully into a 250-mL Erlenmeyer flask and 15-ml 72% sulfuric acid was added. The Erlenmeyer flask was jammed and shaken for 1 min to make the sample wet entirely, after then it was placed under room-temperature for 2.5 h, meantime shaking the Erlenmeyer flask constantly. After 2.5 h, all the substance was transferred into a 1 000-mL Erlenmeyer flask, washing the 250-mL Erlenmeyer flask with distilled water, and making sure that all the residue was transferred into the 1 000-mL Erlenmeyer flask, then adding distilled water to 560 ml. The circumfluence condensator was installed on the 1 000-ml Erlenmeyer flask, seething it for 4 h, then placing the flask some times till the insolubility-substance depositing. The deposition was filtrated with constant weight sand-core filler and put into 105°C drying-oven for drying to constant weight, thus the final increased weight of filler is the weight of Klason lignin.

(2) Determination of total cellulose: The sample was transferred into a 250-mL Erlenmeyer flask by the clean brush, and then 60-mL distilled water, 0.5-mL acetic acid and 0.6-g chlorite sodium (100%) were added, shaking to well-distributed, covering it. Then, the Erlenmeyer flask was put into 75°C shaking bath for 1 h, after then, 0.5-ml acetic acid and 0.6-g chlorite sodium were added, shaking to well-distributed, continue heating 1 h, repeating above operation until the sample color turns to white. Finally they were filtered with constant weight sand-core filler and the residue was dried at 105°C to constant weight. The increased weight of the filler is the weight of the total cellulose.

(3) Determination of the moisture content: Two gram straw powder was put into a flat bottle which is clean and has constant weight and dried at 105°C for 4 h, after then the flat bottle was transferred into dryer to cool for 15 min, then weighting. Again weighted the flat bottle drying-cooling- weight, and repeated the operation until the weight constant. The decreasing weight of the bottle is the

weight of water.

(4) Selection Factor = degradation ratio of Klason lignin / degradation ratio of total cellulose

Results

Screening strains

The strains, which can produce color-zone on the plate of culture medium I, were inoculated on the plate of culture medium II. The result showed that 12 strains produced color-zone on the plate of culture medium II, of which 9 of the strains' were smaller in colony diameter than that of color-zone.

Experiment of color-zone

The result showed that d_1/d_2 of these strains had a little difference, and every strain showed definite degradation ability (Table 1). Their growth ability on the plate was very different. The growth of *Pleurotus ostreatus, P. cornucopiae* and *Flammulina velutipes* were better, and they almost overgrw the whole plate in one week.

Experiment of lignin degradation

The results of lignin degradation and total cellulose degradation were shown in Table 2 and Table 3, respectively. From Table 2 and Table 3, the Selection Factor could be calculated as Table 4. The results showed that all the strains had the ability of lignin degradation, and moreover cellulose could be degraded.

Table 1. Diameters of the color-zone (d_1) and the colony (d_2), and (d_1/d_2)

Strains	d_1 /cm	d_2 /cm	d_1/d_2
Pleurotus ostreatus	60.25	65.25	0.92
P. cetriopileatus	47.5	53	0.90
P. cornucopiae	59.5	62.5	0.95
Hericium erinaceus	32.75	39.85	0.82
Agaricus bisporus	20.25	23.75	0.85
Coprinus comatus	43	36.6	1.17
Pholiota nameko	53.15	42.5	1.25
Flammulina velutipes (yellow)	64	67	0.96
Flammulina velutipes (white)	50	54	0.93
Hohenbuehelia serotina	38	42.75	0.89
Agaricus bla zei	30.2	32	0.94
Pleurotus eryngii var. *nebrodensis*	51.5	50.5	1.02
Tricholama mongolicum	0	0	0

Table 2. Changes of lignin in straw degraded by different strains

Strains	Straw weight before cultured /g	Lignin content before cultured /%	Weight of Lignin before cultured /g	Weight of lignin after cultured /g	Degradation rate /%
Pleurotus. ostreatus	2.0087	24.50	0.4920	0.4042	17.86
P. cetriopileatus	2.0059	24.50	0.4914	0.4651	5.35
P. cornucopiae	2.0004	24.50	0.4901	0.4341	11.43
Hericium erinaceus	2.0068	24.50	0.4917	0.4399	10.53
Agaricus bisporus	2.046	24.50	0.5013	0.4250	15.21
Coprinus comatus	2.0065	24.50	0.4916	0.4150	15.58
Pholiota nameko	2.0045	24.50	0.4911	0.4215	14.17
Flammulina velutipes (yellow)	2.0204	24.50	0.4950	0.4353	12.06
Flammulina velutipes (white)	2.0036	24.50	0.4909	0.4795	2.30
Hohenbuehelia serotina	2.0043	24.50	0.4911	0.4272	13.01
Agaricus blazei	2.0140	24.50	0.4934	0.4118	16.54
Pleurotus eryngii var. *nebrodensis*	2.0010	24.50	0.4902	0.4322	11.83

Table 3. Changes of total cellulose in straw degraded by different strains

Strains	Straw weight before cultured /g	Total content of cellulose before cultured /%	Weight of total cellulose /g		Degradation rate /%
			before cultured	after cultured	
Pleurotus. ostreatus	2.0079	76.16	1.5292	1.4919	2.44
P. cetriopileatus	2.0008	76.16	1.5238	1.4384	5.60
P. cornucopiae	2.0042	76.16	1.5264	1.4294	6.35
Hericium erinaceus	2.0085	76.16	1.5297	1.3284	13.16
Agaricus bisporus	2.02	76.16	1.5384	1.4341	6.78
Coprinus comatus	2.0035	76.16	1.5259	1.3649	10.55
Pholiota nameko	2.0145	76.16	1.5342	1.2685	17.32
Flammulina velutipes (yellow)	2.0492	76.16	1.5607	1.4204	8.99
Flammulina velutipes (white)	2.0052	76.16	1.5272	1.2848	15.87
Hohenbuehelia serotina	2.0166	76.16	1.5358	1.3904	9.47
Agaricus blazei	2.0004	76.16	1.5235	1.4186	6.89
Pleurotus eryngii var. *nebrodensis*	2.0099	76.16	1.5307	1.415	7.56

Table 4. Selection Factor

Strains	Ratio of lignin degradation /%	Ratio of total cellulose degradation /%	Selection Factor
Pleurotus. ostreatus	17.86	2.24	7.97
P. cetriopileatus	5.35	5.60	0.96
P. cornucopiae	11.43	6.35	1.80
Hericium erinaceus	10.53	13.16	0.80
Agaricus bisporus	15.21	6.78	2.24
Coprinus comatus	15.58	10.55	1.48
Pholiota nameko	14.17	17.32	0.82
Flammulina velutipes (yellow)	12.06	8.99	1.34
Flammulina velutipes (white)	2.30	15.87	0.14
Hohenbuehelia serotina	13.01	9.47	1.37
Agaricus bla zei	16.54	6.89	2.40
Pleurotus eryngii var. *nebrodensis*	11.83	7.56	1.56

Relativity test

If SF is *X*, d_1/d_2 is *Y*, then b=-0.00925, a=0.984304, X=1.9067, Y=0.9667

The equation is $Y=0.984304X-0.00925$

By calculating, r= -0.1476, so *X* and *Y* are negative relative, and the relativity is not very significant (Table 5).

Table 5. The relativity of SF and d_1/d_2

SF (*X*)	7.97	0.96	1.8	0.8	2.24	1.48
d1/d2 (*Y*)	0.92	0.9	0.95	0.82	0.85	1.17
SF (*X*)	0.82	1.34	0.14	1.37	2.4	1.56
d1/d2 (*Y*)	1.25	0.96	0.93	0.89	0.94	1.02

Discussion

In the screening of strains and the experiment of lignin biodegradation, it was shown that all the strains except *Tricholama mongolicum* have definite degradation ability to straw lignin, and many strains showed strong degradation ability. In the color-zone experiment, majority strains can degrade lignin firstly, among them *Pleurotus ostreatus, P. cornucopiae,* and *Flammulina velutipes* etc. grew very quickly on the plate, in one week they almost occupy the whole plate; but *P. cetriopileatus, Hericium erinaceus, Coprinus comatus, Flammulina velutipes* and *Hohenbuehelia serotina* grew slowly; *Agaricus bisporus* produce a color-zone on the plate, but the strain did not grow and its growth might be controlled by guaiacol. *Tricholama mongolicum* is an exception, neither grows, nor produces color-zone, and the possible explanation is that it is symbiosis fungus and needs especial nutrition condition. Some strains have priority degradation ability to cellulose, such as *Coprinus comatus* and *Pleurotus eryngii* var. *nebrodensis*. The ratios d_1/d_2 of strains which have priority degradation ability to lignin were all not very high, with a range of 0.8 to 0.95.

In the experiment of lignin degradation, *Pleurotus ostreatus* showed the strongest degradation ability, and the degradation rate of Klasson lignin reached 17.86% after 15 days culture, followed by *Agaricus blazei, Agaricus bisporus* and *Coprinus comatus*, and their degradation ratios of Klasson lignin are 16.54%, 15.21% and 15.58%, respectively. *P. cetriopileatus* and *Flammulina velutipes* showed lower degradation ability to lignin, with a degradation rate of Klasson lignin of 5.35% and 2.30%, respectively, after 15 days culture.

The degradation ability of edible fungi to lignin and straw should be also considered. The ideal strain for straw degradation must have not only the high degradation ability to lignin but also low degradation ability to cellulose. From Table 3, it could be found that *Pleurotus ostreatus* is an ideal strain for straw degradation exactly. Its degradation rate to cellulose only reached 2.44% after cultured for15 days. The other strains have high degradation rate to both lignin and cellulose. The degradation rates to cellulose for *Coprinus comatus, Agaricus blazei* and *Agaricus bisporus* were 6.89%, 6.78% and 10.55%, respectively. *Pholiota mameko* and *Flammulina velutipes* had higher degradation ability to cellulose, compared with other experiment fungi, and their degradation rates reached 17.32% and 15.87%, respectively.

The strain which has high SF is the perfect strain. Table 4 showed that *Pleurotus ostreatus* is an ideal strain, and its SF reach 7.97. The SFs *Coprinus comatus, Agaricus blazei,* and *Agaricus bisporus* were only 1.8, 2.24 and 1.48, respectively.

Linearity regress equation indicated that the relativity of d_1/d_2 and SF is negative correlation, and the correlation coefficient is very low (-0.1476), which showed that the correlation between d_1/d_2 and SF is not very significant.

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